Relative Toxicity of Gossypol Enantiomers in Laying and Broiler Breeder Hens[1,2]

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ABSTRACT Gossypol, a natural component of cottonseed meal, exists in positive (+) or negative (–) enantiomeric forms, and their levels and ratio could be altered by developing new genetic strains of cotton. Two experiments were conducted to determine the relative toxicity of the individual gossypol enantiomers in laying and broiler breeder hens. In the first experiment, 25 individually caged Hy-Line W-36 forty-three-week-old laying hens were fed a standard corn-soy diet supplemented with either no gossypol or the individual enantiomers at 200 and 400 mg/kg of diet for 20 d (5 hens/treatment). In the second experiment, 15 individually caged Cobb 500 fast-feathering 44-wk-old broiler breeder hens were fed a standard corn-soy-wheat middlings diet supplemented with either no gossypol or the individual enantiomers at 400 mg/kg of diet for 18 d (5 hens/treatment). In both experiments, feed intake, egg production, and egg weight were determined daily. All eggs were individually opened and scored for yolk discoloration. At the end of both experiments, several organ and tissue samples were collected for gossypol analyses. In both experiments, the addition of (+)-gossypol to the diet reduced egg production. Only laying and broiler breeder hens fed (+)-gossypol produced eggs with severe yolk discoloration (score ≥ 4). Total feed intake was lower ($P < 0.05$) in laying hens fed the 400 mg/kg level of (+)-gossypol compared with laying hens fed the other dietary treatments. In contrast, broiler breeder hens consumed less of the diet supplemented with (–)-gossypol. In both experiments, tissue accumulation of (+)-gossypol was higher than (–)-gossypol, with the exception of bile and excreta. The results suggest that in hens the ingestion of (+)-gossypol has a greater effect on egg yolk discoloration than the consumption of (–)-gossypol.

Key words: gossypol enantiomer, laying hen, broiler breeder hen, egg, yolk discoloration

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INTRODUCTION

Cottonseed meal (**CSM**) is an attractive alternative protein source for poultry diets, but concern over the presence of gossypol, a potentially toxic agent, has limited its use. Gossypol [1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl-(2,2′-binaphthalene)-8,8′-dicarboxaldehyde] is a polyphenolic compound located predominantly in the lysigenous glands that are distributed throughout the cotton plant (Heinstein et al., 1977). Gossypol is composed of 2 naphthalene rings, and the rotation of these rings around the bond connecting them is restricted. As a result, gossypol occurs naturally as a mixture of 2 enantiomers [(+)- and (–)-gossypol] that differ in their optical properties (Huang et al., 1987). Enantiomers are mirror image forms of the same compound. The chemical and physical properties of the 2 forms are identical, except they rotate the plane of plane-polarized light equally but in opposite directions (Roberts et al., 1971).

Although CSM is substantially lower in energy and protein than soybean meal, it can still be used successfully in poultry diets. Based on its nutrient profile, CSM is a more valuable feed ingredient in laying hen diets than in broiler diets, because laying hens have lower energy and protein requirements than broilers. The laying hen, however, is more sensitive to gossypol ingestion than broilers. Laying hens fed diets containing gossypol can produce eggs that have brown yolk discoloration (Phelps, 1966; Reid et al., 1984; Panigrahi, 1990; Panigrahi and Plumb, 1996; Davis et al., 2002).

The toxic effects of gossypol in poultry diets may be alleviated by the addition of highly soluble Fe salts that bind gossypol (Withers and Brewster, 1917; Gallup, 1928; Eagle, 1949; Panigrahi et al., 1989; Panigrahi and Morris, 1991). Even the gossypol-related brown yolk discoloration of eggs produced by laying hens fed diets containing CSM can be prevented when crystalline ferrous sulphate

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heptahydrate is added to the diet at a 4:1 weight ratio of Fe to free gossypol (Panigrahi, 1990; Panigrahi and Morris, 1991; Panigrahi and Plumb, 1996). However, Fe supplementation is costly, contributes to the heavy metal content of feces, and can depress bird performance by reducing the availability of dietary P (Panigrahi and Plumb, 1996).

Recently, Lordelo et al. (2005) reported that feeding broilers a diet supplemented with (+)-gossypol was less detrimental to broiler performance than feeding a diet containing (−)-gossypol. The results suggested that developing genetic strains of cotton with a high (+)- to (−)-enantiomeric ratio would be beneficial for the utilization of CSM in broiler production. Because CSM is a more advantageous feed ingredient for laying hens than for broilers, the goal of the present research was to determine if feeding (+)- gossypol to laying and broiler breeder hens would have any detrimental effect on egg production and yolk quality.

MATERIALS AND METHODS

Experiment 1

Previous research has indicated that some genetic strains and individual laying hens are less susceptible to the effects of gossypol consumption (Heywang et al., 1954; Panigrahi et al., 1989; Panigrahi and Morris, 1991; Davis et al., 2002). Therefore, initially, 100 individually caged, 32-wk-old, Hy-line W-36 laying hens were fed a standard diet containing 30% CSM (Table 1) for 4 wk to determine their susceptibility to gossypol. The standard CSM diet contained a free gossypol concentration of 220 mg/kg of diet. Hens were given free access to water and the standard CSM diet and maintained on a daily schedule of 17L:7D. Eggs were collected daily from each bird and were stored at 4°C for 14 d to enhance yolk discoloration (Heywang et al., 1954; Phelps, 1966). After storage, eggs were individually opened, and the degree of yolk discoloration was scored based on a previously described scale of 0 to 10 (Davis et al., 2002). Hens that consistently produced eggs with a yolk discoloration score greater than 3 were considered to be gossypol sensitive. Eighty-eight percent of the hens were classified as gossypol-sensitive.

After the laying hens were fed the CSM diet for 4 wk, they were switched to a standard layer diet with 0% CSM (Table 1) for 7 wk. This period allowed for the elimination of gossypol from body tissues (Lordelo et al., 2004). At 43 wk of age, 25 hens were randomly selected from the gossypol-sensitive birds, and these selected birds were weighed and randomly assigned to 5 dietary treatments. Treatments consisted of the standard corn-soy layer diet (Table 1) supplemented with either no gossypol or the individual enantiomers at 200 and 400 mg/kg of diet. Throughout the 20 d experimental period, hens were given free access to water and the mash experimental diets and maintained on their previous daily lighting schedule. The Institutional Animal Care and Use Committee of the University of Georgia approved all animal procedures.

Table 1. Composition of the experimental diets (experiments 1 and 2)

	Diet (%)		
Ingredient	CSM layer[1]	Layer[2]	Broiler breeder[3]
Corn	53.78	61.75	63.77
Soybean meal, 48% CP	0.00	26.02	20.74
Cottonseed meal	30.00	0.00	0.00
Wheat middlings	—	—	4.30
Limestone	9.06	8.98	7.97
Dicalcium phosphate	1.15	1.29	1.39
Poultry fat	4.60	1.15	1.00
Salt	0.44	0.42	0.40
Vitamin mix[4]	0.25	0.25	0.30
DL-Met	0.12	0.06	0.07
Mineral mix[5]	0.06	0.06	0.06
L-Lys, HCl	0.54	0.02	0.00
Calculated analysis[6]			
ME (kcal/kg)	2,840.00	2,840.00	2,850.00
CP (%)	17.30	17.30	15.50
Ca (%)	3.70	3.70	3.40
Available P (%)	0.38	0.38	0.37
Met and cystine (%)	0.66	0.66	0.60
Lys (%)	0.95	0.95	0.79

[1]Cottonseed meal (CSM; 30%) layer diet utilized in experiment 1 to determine the susceptibility of hens to gossypol.

[2]Layer diet utilized in experiment 1.

[3]Broiler breeder diet utilized in experiment 2.

[4]Vitamin mix provided the following per kilogram of diet: vitamin A, 5,510 IU; vitamin D_3, 1,100 IU; vitamin E, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.4 mg; niacin, 44.1 mg; D-pantothenic acid, 11.2 mg; choline, 191.3 mg; menadione sodium bisulfate, 3.3 mg; folic acid, 5.5 mg; pyridoxine HCl, 4.7 mg; thiamin, 2.2 mg; D-biotin, 0.11 mg; and ethoxyquin, 125 mg.

[5]Mineral mix provided the following in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; I, 1.5; and Se, 0.5.

[6]Calculated analysis was based on Dale (2001).

The individual gossypol enantiomers were prepared by crystallization as previously described (Dowd, 2003) and were at least 99.5% optically pure based on HPLC analysis. It was previously determined by Lordelo et al. (2005) that the gossypol enantiomers remained free (i.e., unbound) in feed for at least 24 h. Hence, each of the gossypol-supplemented diets was mixed on a daily basis to minimize the potential of gossypol binding to other feed components. Feed consumption and mortality were recorded daily and BW was determined on d 1 and 20 of the study.

Excreta were collected from each bird for the last 24 h of the experiment. At the end of the 20-d experimental period, blood was collected from the brachial vein and placed in heparinized glass tubes on ice. The samples were then centrifuged for 10 min at 3,000 × *g*, and the collected plasma from each sample was frozen at −80°C for future gossypol analyses. In addition, blood was collected in 2 heparinized capillary tubes from the brachial vein of each bird. The capillary tubes were immediately centrifuged in a microcapillary centrifuge, and the percentage of packed cell volume was determined with a microcapillary reader (International Equipment Co., Needham Heights, MA).

Table 2. Egg production of hens fed diets with (+)- or (–)-gossypol during successive 5-d experimental periods[1] (experiment 1)

Dietary gossypol (mg/kg)	1 to 5 d	6 to 10 d	11 to 15 d	16 to 20 d	0 to 20 d
	(number of eggs/bird)				
0	4.8 ± 0.2	4.2 ± 0.2[a]	4.2 ± 0.2[a]	4.6 ± 0.2[a]	17.8 ± 0.4[a]
200 (–)	4.6 ± 0.4	3.8 ± 0.2[a]	4.0 ± 0.0[a]	4.2 ± 0.2[ab]	16.6 ± 0.5[a]
400 (–)	4.8 ± 0.2	4.0 ± 0.3[a]	4.2 ± 0.2[a]	4.4 ± 0.2[a]	17.4 ± 0.4[a]
200 (+)	4.8 ± 0.2	3.8 ± 0.2[a]	3.3 ± 0.8[ab]	2.7 ± 0.6[b]	14.5 ± 1.2[a]
400 (+)	4.0 ± 0.5	0.4 ± 0.4[b]	1.2 ± 0.7[b]	0.4 ± 0.4[c]	6.0 ± 1.8[b]

[a,b]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per dietary treatment. There were 5 replicate birds for each dietary treatment except for the 200 (+) mg/kg treatment, in which n = 4 replicate birds.

Birds were killed by cervical dislocation and the liver, heart, right kidney, spleen, and portions (~3 g) of abdominal fat and the left pectoralis major muscle were collected. Contents of the gallbladder of each bird were collected with a needle and syringe. The developing preovulatory follicles were visually examined for abnormalities. The individual organs, tissues, and excreta were weighed and immediately frozen at –80°C for future gossypol analyses.

Egg production and egg weight were determined daily. Eggs were stored for 14 d at 4°C, and the yolks were scored for discoloration as previously described (Davis et al., 2002). The yolk and albumen of each egg were then separated, placed in individual containers, and frozen at –80°C for future gossypol analyses. The egg data were summarized in 5-d intervals throughout the experiment to facilitate the analysis of the data by avoiding daily fluctuations in egg production in treatment groups that consisted of only 5 birds.

Experiment 2

To determine if broiler breeder hens responded to the ingestion of the individual enantiomers of gossypol in a similar manner as laying hens, 15 individually caged Cobb 500 fast-feathering 44-wk-old broiler breeder hens were randomly assigned to 3 dietary treatments. The treatments consisted of a standard corn-soy-wheat middlings breeder diet (Table 1) supplemented with either no gossypol or each gossypol enantiomer at 400 mg/kg of diet. The broiler breeder hens were maintained on a daily lighting schedule of 16L:8D. Each broiler breeder hen was fed 145 g of feed per day as recommended by the *Cobb 500 Breeder Management Guide* (Cobb-Vantress, 2002). Egg production, egg weight, mortality, and feed intake were determined daily for 18 d. Eggs were collected daily and processed as described in experiment 1. At the end of the 18-d experimental period, the birds were killed, and the liver, heart, spleen, ovary, and oviduct were collected from each bird. The contents of the gallbladder of each bird were collected with a needle and syringe. The individual organs were weighed and immediately frozen at –80°C for future gossypol analyses. Plasma and packed cell volume were obtained and processed as described in experiment 1.

Gossypol Determination

Tissues were freeze-dried for 48 h (which was sufficient to reach a constant dry weight). The concentration of (+)- and (–)-gossypol in organs, tissues, excreta, egg yolk, and albumen was determined by HPLC as previously described (McMillan, 2000). The free gossypol content of the CSM used for the laying hen diet was determined as previously described (Davis et al., 2002).

Statistical Analyses

Data from each experiment were subjected to ANOVA according to the GLM procedure. Tukey's multiple-com-

Table 3. Average egg weight during successive 5-d experimental periods for laying hens fed (+)- or (–)-gossypol for 20 d (experiment 1)[1]

Dietary gossypol (mg/kg)	1 to 5 d	6 to 10 d	11 to 15 d	16 to 20 d	0 to 20 d
	(g)				
0	61.4 ± 2.7	60.8 ± 2.3	59.3 ± 2.4[a]	58.8 ± 2.2[a]	60.1 ± 2.4[ab]
200 (–)	62.5 ± 0.8	60.6 ± 0.9	61.3 ± 0.9[a]	61.1 ± 0.5[a]	61.4 ± 0.8[a]
400 (–)	60.1 ± 1.3	57.7 ± 0.9	59.3 ± 1.5[a]	57.8 ± 1.0[a]	58.8 ± 1.2[ab]
200 (+)	59.4 ± 2.0	55.4 ± 1.0	51.7 ± 1.7[b]	49.9 ± 1.5[b]	54.1 ± 1.6[bc]
400 (+)	58.1 ± 2.4	57.7 ± 1.4	47.0 ± 2.6[b]	42.4 ± 0.0[c]	51.3 ± 1.6[c]

[a–c]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per dietary treatment. There were 5 replicate birds for each dietary treatment except for the 200 (+) mg/kg treatment, in which n = 4 replicate birds.

Table 4. Average egg yolk score and percentages of objectionable egg yolks produced by laying hens fed individual pure gossypol enantiomers during successive 5-d experimental periods[1] (experiment 1)

Dietary gossypol (mg/kg)	1 to 5 d			6 to 10 d			11 to 15 d			16 to 20 d		
	Score	OBJ (%)	n	Score	OBJ (%)	n	Score	OBJ (%)	n	Score	OBJ (%)	n
0	0.90 ± 0.06[b]	0	24	0.90 ± 0.07[c]	0	21	0.80 ± 0.09[d]	0	20	0.92 ± 0.08[d]	0	25
200 (–)	1.32 ± 0.10[b]	0	22	1.41 ± 0.17[bc]	6	18	1.79 ± 0.16[c]	16	19	1.42 ± 0.10[d]	0	26
400 (–)	1.71 ± 0.11[b]	4	24	1.85 ± 0.17[b]	20	20	2.14 ± 0.12[c]	24	21	2.08 ± 0.15[c]	31	26
200 (+)	1.70 ± 0.15[ab]	5	20	3.79 ± 0.56[a]	80	15	5.29 ± 0.46[b]	100	14	5.83 ± 0.45[b]	100	11
400 (+)	2.70 ± 0.56[a]	30	20	3.50 ± 1.50[ab]	50	2	8.00 ± 0.84[a]	100	6	9.50 ± 0.50[a]	100	2

[a–d]Values within a column without a common superscript differ; $P < 0.05$.

[1]Egg yolks were scored (scale 0 to 10) individually for discoloration as previously described (Davis et al., 2002). Values are means ± SEM per egg yolk. The percentage of the egg yolks with an objectionable (OBJ) score (Davis et al., 2002) was calculated by dividing the number of yolks with a score greater than or equal to 3 by the total number of yolks examined (n) within each period for each dietary treatment. The number of egg yolks analyzed (n) for each period varied due to differences in egg production for the dietary treatments and the incidence of double-yolked eggs.

parison procedure was used to detect significant differences among diets. All statistical procedures were done with a SAS statistical software package (SAS Institute, 2001), and differences were considered significant when *P*-values were less than 0.05.

RESULTS

Experiment 1

Feeding hens either level of (–)-gossypol did not significantly affect egg production (Table 2). For the period of 16 to 20 d, egg production was less ($P < 0.05$) for hens fed the 200 mg/kg (+)-gossypol diet compared with hens fed the control diet. After the first 5-d period, egg production was less ($P < 0.05$) for the hens receiving the 400 mg/kg (+)-gossypol diet than for hens fed the control diet or those fed diets containing (–)-gossypol (Table 2). Initially, egg weight was not affected by the inclusion of either enantiomer in the diets, but eggs produced by birds fed (+)-gossypol weighed less than those produced by the birds fed the control diet or the diets containing (–)-gossypol during the last half of the experiment (Table 3). During the period from 16 to 20 d, eggs from the hens fed the 400 mg/kg (+)-gossypol diet weighed less than eggs from those fed the 200 mg/kg (+)-gossypol diet (Table 3).

At the end of the experiment, the ovary of each hen was visually examined. Sixty percent of the birds fed the 400 mg of (+)-gossypol/kg of diet had 1 or more atretic hierarchical preovulatory follicles (data not shown). In contrast, there were no visible signs of follicular atresia in the hierarchal follicles of birds fed any of the other dietary treatments.

Hens fed the 400 mg/kg diet of (+)-gossypol produced egg yolks that had a significantly elevated discoloration score for each of the experimental periods when compared with the egg yolks produced from hens fed the control diet (Table 4). Ingestion of (+)-gossypol caused hens to produce eggs with a high incidence of objectionable egg yolk discoloration (Table 4). Of the objectionable eggs produced by hens fed (+)-gossypol, 75% had severe discoloration (score of 4 to 10; data not shown). In contrast, the objectionable egg yolks produced by the hens fed either level of (–)-gossypol never had a score greater than 3 (data not shown).

Only the gossypol enantiomer fed was detected in egg yolks. No gossypol was detected in the albumen of any of the eggs produced from birds fed either of the gossypol enantiomers (data not shown). During the period of 1 to 5 d, the concentration of gossypol in the yolk of eggs produced by hens fed either level of (+)-gossypol was not significantly different from the concentration of gossypol in the yolk of eggs produced by hens fed either level of (–)-gossypol (Table 5). The limited number of yolk samples analyzed after the initial period for the 400 mg (+)-gossypol treatment precluded making meaningful

Table 5. Average concentration of (+)- and (–)-gossypol (mg/kg of DM) in the yolk of eggs produced by laying hens fed individual pure gossypol enantiomers during successive 5-d experimental periods[1] (experiment 1)

Dietary gossypol (mg/kg)	1 to 5 d		6 to 10 d		11 to 15 d		16 to 20 d	
	mg/kg of DM	n	mg/kg of DM	n	mg/kg of DM	n	mg/kg of DM	n
200 (–)	61 ± 12	22	189 ± 5[c]	18	211 ± 6[d]	19	235 ± 7[b]	26
400 (–)	117 ± 19	24	324 ± 10[a]	20	379 ± 9[b]	21	386 ± 6[a]	26
200 (+)	59 ± 14	20	254 ± 10[b]	15	325 ± 9[c]	14	365 ± 13[a]	11
400 (+)	86 ± 20	20	286 ± 78[ab]	2	474 ± 20[a]	6	414 ± 43[a]	2

[a–d]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per egg yolk; n = the number of egg yolks analyzed, and this value varied due to differences in egg production for the dietary treatments and the incidence of double-yolked eggs. No gossypol was detected in the yolks of birds fed 0 mg of gossypol/kg of diet; (–)-gossypol was not detected in egg yolks of hens fed (+)-gossypol, and (+)-gossypol was not detected in those fed (–)-gossypol.

Table 6. Body weight change and feed consumption of laying hens fed (+)- or (–)-gossypol for 20 d[1] (experiment 1)

Dietary gossypol (mg/kg)	BW change (g)	Total feed consumption (g)
0	–54 ± 26[ab]	1,802 ± 59[a]
200 (–)	–54 ± 9[ab]	1,723 ± 71[a]
400 (–)	–94 ± 38[b]	1,642 ± 55[a]
200 (+)	–22 ± 25[ab]	1,697 ± 63[a]
400 (+)	8 ± 29[a]	1,343 ± 68[b]

[a,b]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per bird for the 20-d experimental period, with 5 replicate birds for each dietary treatment except for the 200 (+) mg/kg treatment, in which n = 4 replicate birds.

comparisons with other treatments. In all but the initial period, gossypol levels were higher in the yolk of eggs produced by hens fed the diet containing 400 mg/kg of (–)-gossypol compared with the yolk of the eggs produced by hens fed the diet containing 200 mg/kg of (–)-gossypol. After the initial period, gossypol levels were higher in yolks of hens fed 200 mg of (+)-gossypol than in yolks of those fed the same level of (–)-gossypol (Table 5).

The mean initial BW for all the hens was 1,622 g. Change in BW for the hens fed diets supplemented with gossypol was not different from the change in BW for the hens fed the control diet (Table 6). However, loss in BW was greater for hens receiving the 400 mg (–)-gossypol diet than for hens fed the 400 mg (+)-gossypol diet (Table 6). Total feed consumption was less ($P < 0.05$) for hens fed the 400 mg/kg (+)-gossypol diet compared with the controls and all other gossypol treatments (Table 6). One bird from the group fed the diet containing 200 mg/kg of (–)-gossypol died on d 19. A necropsy of this hen revealed that it had a large solid tumor in its abdominal cavity. The data collected from this bird were not included in the results.

Compared with birds fed all other treatments, liver and spleen weights relative to BW were increased ($P < 0.05$) in hens fed the diet containing the highest concentration of (+)-gossypol (Table 7). The weight of the heart and kidney as well as total bile volume relative to BW were not affected by the ingestion of either gossypol enantiomer (Table 7). Percentage of packed blood cell volume was not different ($P > 0.05$) among birds fed any of the dietary treatments (data not shown).

In laying hens fed (–)-gossypol, excreta had the highest concentration of gossypol, followed by liver, bile, kidney, plasma, spleen, heart, and muscle (Table 8). In contrast, the liver had the highest accumulation of gossypol in birds fed (+)-gossypol, followed by the excreta, kidney, spleen, plasma, bile, heart, and muscle (Table 8). Interestingly, neither (+)- or (–)-gossypol was detected in any of the collected fat samples (data not shown). For a given dietary concentration of gossypol, the accumulation of (+)-gossypol was higher ($P < 0.05$) than that of (–)-gossypol in all of the tissues examined, except for the bile. The response for bile was similar to the other tissues, but the differences were not significant (Table 8). In contrast to the tissues, gossypol levels in excreta were higher in hens fed (–)-gossypol (Table 8). Only the gossypol enantiomer that was fed was detected in the tissues analyzed.

Experiment 2

During the last half of the experiment, there was a decrease in egg production in birds fed either gossypol enantiomer (Table 9). Furthermore, no eggs were produced by the broiler breeder hens fed (+)-gossypol after d 15 (data not shown). For the entire experiment, total egg production was depressed in the birds fed the diet containing (+)-gossypol compared with the control birds (Table 9).

Egg weight was not affected by feeding (–)-gossypol, but egg weight was decreased ($P < 0.05$) during the 10- to 18-d period in birds fed (+)-gossypol (data not shown). However, because of the low production of eggs from hens fed (+)-gossypol, only 4 eggs were available for comparison.

Hens fed the 400 mg/kg diet of (+)-gossypol produced egg yolks that had a significantly elevated discoloration score for each of the experimental periods when compared with the egg yolks produced from hens fed either the control or the diet containing 400 mg/kg of (–)-gossypol (Table 10). None of the yolks from eggs produced by the hens fed (–)-gossypol had a score greater than 4 (data not shown). The accumulation of gossypol in the yolks was similar for (+)- and (–)-gossypol (Table 10). However, the accumulation of (+)-gossypol in the yolk material

Table 7. Organ weights for laying hens fed (+)- or (–)-gossypol for 20 d[1] (experiment 1)

Dietary gossypol (mg/kg)	Heart	Liver	Kidney	Spleen	Bile
	(g/100 g of BW)				(mL/100 g of BW)
0	0.40 ± 0.03	2.04 ± 0.08[b]	0.29 ± 0.02[ab]	0.09 ± 0.00[b]	0.07 ± 0.01
200 (–)	0.39 ± 0.04	2.14 ± 0.11[b]	0.27 ± 0.01[b]	0.08 ± 0.01[b]	0.08 ± 0.01
400 (–)	0.41 ± 0.02	2.41 ± 0.13[b]	0.32 ± 0.01[a]	0.09 ± 0.01[b]	0.07 ± 0.01
200 (+)	0.44 ± 0.04	2.30 ± 0.09[b]	0.29 ± 0.02[ab]	0.09 ± 0.01[b]	0.09 ± 0.01
400 (+)	0.43 ± 0.02	2.93 ± 0.23[a]	0.34 ± 0.02[a]	0.21 ± 0.03[a]	0.08 ± 0.02

[a,b]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per bird for the 20-d experimental period, with 5 replicate birds for each dietary treatment except for the 200 (+) mg/kg treatment, in which n = 4 replicate birds.

Table 8. Concentrations of (+)- and (–)-gossypol in tissues and excreta from laying hens fed pure individual gossypol enantiomers for 20 d[1] (experiment 1)

Dietary gossypol (mg/kg)	Liver	Spleen	Kidney	Heart	Muscle	Excreta	Plasma	Bile
	(mg/kg of DM)						(mg/L)	
200 (–)	243 ± 39[d]	31 ± 5[d]	43 ± 4[d]	7 ± 2[c]	6 ± 1[c]	861 ± 136[b]	33 ± 7[c]	102 ± 7
400 (–)	477 ± 60[c]	54 ± 4[c]	92 ± 8[c]	21 ± 7[bc]	11 ± 1[b]	1,646 ± 188[a]	70 ± 14[b]	138 ± 7
200 (+)	930 ± 117[b]	108 ± 6[b]	145 ± 15[b]	34 ± 6[b]	13 ± 2[b]	465 ± 111[c]	79 ± 6[b]	109 ± 10
400 (+)	1,852 ± 118[a]	283 ± 41[a]	397 ± 72[a]	55 ± 5[a]	33 ± 4[a]	681 ± 80[bc]	232 ± 2[a]	167 ± 36

[a–d]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per bird for the 20-d experimental period, with 5 replicate birds for each dietary treatment except for the 200 (+) mg/kg treatment, in which n = 4 replicate birds. No gossypol was detected in the tissues of birds fed 0 mg of gossypol/kg of diet; (–)-gossypol was not detected in tissues of hens fed (+)-gossypol, and (+)-gossypol was not detected in those fed (–)-gossypol.

collected at the end of the experiment from the F_1 to F_4 follicles of hens fed (+)-gossypol was greater ($P < 0.05$) than the accumulation of (–)-gossypol in the yolk material of hens fed (–)-gossypol (721.9 ± 9.8 vs. 413.1 ± 6.8 mg/kg of DM).

At the end of the experiment, 80% of birds fed (+)-gossypol had 1 or more atretic large hierarchical preovulatory follicles (data not shown). The remaining follicles tended to be small in size and contained dark, discolored yolk material. In contrast, there were no visible signs of follicular atresia in the hierarchal follicles of birds fed any of the other dietary treatments. Three of the 5 broiler breeder hens fed the diet containing (–)-gossypol had no hierarchical follicles and had small regressed oviducts. These 3 birds were also actively molting feathers. The other 2 birds had a normal hierarchy of preovulatory follicles, and their oviducts were normal in size.

Although the broiler breeder hens in this experiment received a restricted amount of daily feed, as recommended by the breeder guidelines, feeding a diet containing (–)-gossypol significantly ($P < 0.05$) reduced feed intake in these birds. Total feed consumption (mean ± SEM) per hen was 2,590 ± 3.0, 2,182 ± 140, and 2,548 ± 26 g for the hens fed the diets containing 0, 400 (–) or 400 (+) mg of gossypol/kg of diet, respectively. The total amount of feed given to each hen during the experiment was 2,610 g. Therefore, the birds fed the 0 and 400 mg/kg (+)-gossypol diets consumed almost all of their daily allotment of feed, which resulted in very low SEM values for the total feed consumed per bird with these 2 treatments.

The BW change (mean ± SEM) per hen was 157 ± 36, –59 ± 98, and 253 ± 49 g for the hens fed the diets containing 0, 400 (–), or 400 (+) mg of gossypol/kg of diet. Although the change in weight in hens fed gossypol were not significantly different from the control hens, the difference in BW change between the hens fed (–)-gossypol and those fed (+)-gossypol was significant ($P < 0.05$). The initial BW (mean ± SEM) per hen was 3,671 ± 78 g.

Broiler breeder hens fed a diet with 400 mg of (+)-gossypol had increased ($P < 0.05$) liver and spleen weights relative to BW compared with the controls (Table 11). The weight of the ovary relative to BW was lower ($P < 0.05$) for hens fed the diet containing 400 mg/kg of (–)-gossypol compared with hens fed the control diet. The weights of heart, oviduct, and bile were not affected by the treatments (Table 11).

Concentrations of gossypol in liver, spleen, heart, and plasma were higher ($P < 0.05$) for broiler breeder hens fed 400 mg of (+)-gossypol than those fed 400 mg of (–)-gossypol (Table 12). Concentrations of gossypol in bile were not different for the 2 gossypol enantiomers. In broiler breeder hens fed (–)-gossypol, the liver had the greatest accumulation of gossypol, followed by the bile, spleen, heart, and plasma (Table 12). When hens were fed (+)-gossypol, the liver accumulated the most gossypol, followed by the spleen, bile, heart, and plasma (Table 12). Percentages of packed blood cells were not affected by the dietary treatments (data not shown).

DISCUSSION

Based on feeding broilers diets supplemented with pure gossypol enantiomers, Lordelo et al. (2005) suggested that the development of a genetic strain of cotton with only (+)-gossypol would be beneficial for the utilization of CSM by the poultry industry. In addition, the insecticidal properties of gossypol that make it essential for optimal cotton production can apparently be met with just (+)-gossypol (Stipanovic et al., 2006). However, the present study indicates that CSM made from a strain of cotton with only (+)-gossypol could not be universally

Table 9. Egg production for broiler breeder hens fed either (+)- or (–)-gossypol during several experimental periods[1] (experiment 2)

Dietary gossypol (mg/kg)	1 to 9 d	10 to 18 d	1 to 18 d
		(number of eggs/bird)	
0	5.8 ± 0.4	6.2 ± 0.4[a]	12.0 ± 0.6[a]
400 (–)	4.8 ± 0.9	2.8 ± 1.7[b]	7.6 ± 2.5[ab]
400 (+)	4.4 ± 0.5	0.8 ± 0.5[b]	5.2 ± 0.9[b]

[a,b]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per dietary treatment, with n = 5 replicate birds per dietary treatment.

Table 10. Average yolk score, incidence of objectionable yolks, and average concentration of (+)- and (–)-gossypol (mg/kg of DM) in the yolk of eggs produced by broiler breeder hens fed individual pure gossypol enantiomers during successive experimental periods[1] (experiment 2)

Dietary gossypol (mg/kg)	1 to 9 d				10 to 18 d			
	Score	OBJ (%)	mg/kg of DM	n	Score	OBJ (%)	mg/kg of DM	n
0	1.14 ± 0.12[b]	0	Undetectable	28	1.14 ± 0.10[c]	0	Undetectable	27
400 (–)	1.91 ± 0.23[b]	33	108 ± 28.6[a]	24	2.93 ± 0.19[b]	69	320 ± 18.7[a]	16
400 (+)	3.00 ± 0.36[a]	41	106 ± 25.7[a]	22	7.25 ± 1.38[a]	100	364 ± 53.0[a]	4

[a–c]Values within a column without a common superscript differ ($P < 0.05$).

[1]Egg yolks were scored individually for discoloration as previously described (Davis et al., 2002). Values are means ± SEM per egg yolk. The percentage of the egg yolks with an objectionable (OBJ) score (Davis et al., 2002) was calculated by dividing the number of yolks with a score greater than or equal to 3 by the total number of yolks examined (n) within each period for each dietary treatment. The number of egg yolks analyzed (n) for each period varied due to differences in egg production for the dietary treatments and the incidence of double-yolked eggs. Additionally, (–)-gossypol was not detected in egg yolks of hens fed (+)-gossypol, and (+)-gossypol was not detected in those fed (–)-gossypol.

utilized by the poultry industry, because (+)-gossypol caused egg yolk discoloration and decreased feed intake and egg production in laying hens. The CSM produced from cotton varieties that only contained (+)-gossypol in the seed would be more suitable in broiler production (Lordelo et al., 2005), whereas CSM produced from cotton varieties that contained only (–)-gossypol in the seed would be more suitable for laying hens. However, this would require that seed processors be willing to identity preserve lots of CSM, which is not the current practice.

The sensitivity of laying hens to the ingestion of (+)-gossypol was unexpected. In rats (Wang et al., 1987) and broilers (Lordelo et al., 2005), only (–)-gossypol causes severe toxic effects. Furthermore, (–)-gossypol is responsible for the well-documented antifertility (Wang et al., 1987), antiviral (Lin et al., 1989), and anticancer (Liu et al., 2002) effects of gossypol. Our results suggest, however, that (+)-gossypol could have beneficial therapeutic properties as well, because it can also interfere with normal physiological functions in situations in which (–)-gossypol has little or no effect.

Traditionally, gossypol has been assumed to cause egg yolk discoloration based on a chemical combination of gossypol with ferric Fe released from yolk proteins (Swensen et al., 1942; Kemmerer et al., 1961, 1966). In the present research, laying hens only produced egg yolks with severe discoloration (discoloration score ≥ 4) when they were fed diets containing the (+) enantiomer of gossypol. In fact, during the 11- to 15-d experimental period, the hens fed 400 mg/kg of (–)-gossypol had a significantly higher yolk concentration of (–)-gossypol than the concentration of (+)-gossypol in the yolks produced from hens fed 200 mg/kg of (+)-gossypol (Table 5). Nevertheless, the incidence of objectionable egg yolk discoloration was 100% in the yolks produced from the hens fed (+)-gossypol and only 24% in the yolks from hens fed 400 mg/kg of (–)-gossypol (Table 4). Furthermore, the range of the yolk discoloration scores was 1 to 3 for the hens fed the 400-mg (–)-gossypol dietary treatment and was 3 to 9 for the hens fed the 200-mg (+)-gossypol dietary treatment. Both (–)- and (+)-gossypol are capable of binding Fe, and thus the greater potency of (+)-gossypol to cause egg yolk discoloration indicates that the mechanisms by which gossypol causes egg yolk discoloration are more complicated than originally believed (Swensen et al., 1942; Kemmerer et al., 1961, 1966).

In the eggs produced from laying and broiler breeder hens, the yolks contained high levels of the individual gossypol enantiomers, whereas no gossypol was detected in the albumen. These results strongly suggest that the gossypol in egg yolk was probably associated with the very low density lipoprotein or vitellogenin portion of the yolk that is synthesized in the liver and transported to the developing follicles for receptor-mediated uptake rather than being associated with the small fraction of yolk material that is derived directly from the uptake of plasma components by passive diffusion or active transport. Although the yolk of eggs produced by hens fed

Table 11. Organ weights for broiler breeder hens fed (+)- or (–)-gossypol for 18 d[1] (experiment 2)

Dietary gossypol (mg/kg)	Heart	Liver	Spleen	Ovary	Oviduct	Bile
	(g/100 g of BW)					(mL/100 g of BW)
0	0.40 ± 0.04	1.35 ± 0.06[b]	0.06 ± 0.01[b]	1.31 ± 0.06[a]	1.57 ± 0.04	0.05 ± 0.01
400 (–)	0.40 ± 0.04	1.23 ± 0.08[b]	0.08 ± 0.01[ab]	0.58 ± 0.30[b]	1.05 ± 0.44	0.07 ± 0.01
400 (+)	0.39 ± 0.02	1.71 ± 0.09[a]	0.09 ± 0.01[a]	0.95 ± 0.23[ab]	1.50 ± 0.30	0.06 ± 0.01

[a,b]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per bird for the 18-d experimental period, with 5 replicate birds for each dietary treatment.

Table 12. Concentration of (+)- and (–)-gossypol in tissues from broiler breeder hens fed pure individual gossypol enantiomers for 18 d[1] (experiment 2)

Dietary gossypol (mg/kg)	Liver	Spleen	Heart	Plasma	Bile
	(mg/kg of DM)			(mg/L)	
400 (–)	567 ± 83[b]	88 ± 14[b]	74 ± 13[b]	28 ± 2[b]	173 ± 30
400 (+)	1,702 ± 78[a]	237 ± 13[a]	125 ± 4[a]	45 ± 2[a]	185 ± 34

[a,b]Values within a column without a common superscript differ ($P < 0.05$).

[1]Values are means ± SEM per bird for the 18-d experimental period, with 5 replicate birds for each dietary treatment. No gossypol was detected in the tissues of birds fed 0 mg of gossypol/kg of diet; (–)-gossypol was not detected in tissues of hens fed (+)-gossypol, and (+)-gossypol was not detected in those fed (–)-gossypol.

gossypol contained substantial levels of gossypol, neither gossypol enantiomer was detected in the abdominal fat from these hens. Because de novo fatty acid synthesis occurs almost exclusively in the liver of birds (Goodridge and Ball, 1967; Goodridge, 1968; Leveille et al., 1968), these results might indicate that gossypol is transported to the egg yolk from the liver, associated with vitellogenin, or is only incorporated into the specialized yolk-targeted very low density lipoprotein particles (Walzem et al., 1999) destined for uptake by only the growing preovulatory follicles of the hen ovary. More research is needed to determine the mechanisms responsible for the deposition of gossypol in egg yolk.

Previous research indicated that feeding laying hens high levels of CSM reduces egg weight (Heywang et al., 1950; Narain et al., 1957; Reid et al., 1987; Davis et al., 2002). The reduction in egg weight seen previously when feeding CSM probably resulted from the ingestion of (+)-gossypol contained within the CSM, because only the ingestion of (+)-gossypol resulted in laying and broiler breeder hens producing smaller eggs in the current research. However, it is possible that feeding the (–)-enantiomer of gossypol for a longer period or at a higher level in the diet than was done in the current research might also cause a reduction in egg size.

Variation was seen between the laying and broiler breeder hens in their feed consumption response to the gossypol enantiomers. The feed consumption of broiler breeder hens was negatively affected by (–)-gossypol, which agreed with a previous finding in young broilers (Lordelo et al., 2005). In contrast, the feed consumption of laying hens was not affected by the addition of the (–)-enantiomer to the diet, but there was a significant decrease in feed consumption in laying hens fed (+)-gossypol. It is unclear if the reduced feed intake in the laying hens fed (+)-gossypol was a direct or an indirect effect related to their reduced production energy needs as egg production decreased. It appears to be a direct effect, because feed consumption was significantly decreased by d 4. Additionally, egg production did not decrease until d 7 (data not shown). The net effect for the laying hens fed 400 mg/kg of (+)-gossypol was that their decrease in egg production allowed them to maintain their BW even though they ate significantly less feed than the control hens (Table 6). The contradictory results between the feed intake response of laying hens and meat-type birds to the different gossypol enantiomers may be explained by genetic differences. There are previous reports indicating that the feed intake response of chickens to gossypol in CSM (therefore, a mixture of both enantiomers) varied with breed (Heywang et al., 1954; Panigrahi et al., 1989; Panigrahi and Morris, 1991).

In broiler breeder hens, egg production was reduced by feeding either (+)- or (–)-gossypol, but the cause for the decline in production appeared to vary. The reduced egg production for broiler breeder hens fed (–)-gossypol did not occur until the last half of the experimental period. Three of the 5 hens fed the diet containing (–)-gossypol were molting by the end of the experimental period, and these 3 birds had regressed ovaries and oviducts. The significant decrease in feed intake associated with feeding (–)-gossypol may have triggered these hens to molt. In contrast, feeding broiler breeder hens (+)-gossypol rapidly decreased egg production, and, in fact, egg production in these hens completely ceased by the end of the experiment. When the hens fed (+)-gossypol were killed at the end of the experiment, their oviducts were equal in weight to those of the control birds, but their developing preovulatory follicles contained very dark, discolored yolk. Additionally, 4 out of the 5 hens had at least 1 large (F_1-F_4) regressing preovulatory follicle. Furthermore, a similar decrease in egg production and incidence of follicular atresia was observed in laying hens fed 400 mg/kg of (+)-gossypol. Thus, (+)-gossypol may interfere with the normal maturation and ovulation of preovulatory follicles, which subsequently results in decreased egg production.

In the present research, accumulation of (+)-gossypol was always more than twice that of (–)-gossypol in the tissues examined from laying and broiler breeder hens. This difference in gossypol enantiomer accumulation was also reported in broilers (Lordelo et al., 2005). The biological mechanisms associated with the differences in the tissue accumulation of the 2 gossypol enantiomers are unclear. However, the accumulation of (–)-gossypol in the bile and egg yolks from hens fed (–)-gossypol was similar to the levels of (+)-gossypol found in the bile and egg yolks from hens fed (+)-gossypol. Bile volume was equal in the hens fed both isomers of gossypol. Although egg production was lower in hens fed 400 mg/kg of (+)-gossypol, there was no difference in egg production in the laying hens fed either 200 mg/kg of (+)- or (–)-gossypol.

Therefore, based on this limited information, it appears that the elimination of (+)- and (–)-gossypol in the hen through bile and eggs appear to be similar and probably cannot account for the differences in tissue accumulation of the 2 enantiomers.

Gossypol accumulation in excreta, however, was much higher in laying hens fed the diets containing (–)-gossypol than in those fed the diets containing (+)-gossypol. Total fecal output from the laying hens fed the different dietary treatments was equal during the 24-h collection period. Thus, the results suggest that in laying hens, (–)-gossypol is either absorbed from the intestine at a lower rate than (+)-gossypol or is excreted by the kidneys at a higher rate than (+)-gossypol (because the fecal samples contain the urinary wastes as well). Additional research is needed on the intestinal absorption and kidney excretion of the gossypol enantiomers in chickens to determine which might contribute to the observed differences in the tissue accumulation of the 2 enantiomers.

In summary, both (+) and negative (–) forms of gossypol can cause detrimental effects in hens. Severe egg yolk discoloration was only caused by feeding hens (+)-gossypol, and the mechanism by which (+)-gossypol caused this yolk discoloration is unknown. Finally, the greater tissue accumulation of (+)-gossypol than (–)-gossypol in hens may be related to differences in kidney excretion, intestinal absorption of the 2 enantiomers of gossypol, or both.

REFERENCES

Cobb-Vantress. 2002. Cobb 500 Breeder Management Guide. Cobb-Vantress Inc., Siloam Springs, AR.

Dale, N. M. 2001. Feedstuffs ingredient analysis table. Feedstuffs 73:28–37.

Davis, A. J., M. M. Lordelo, and N. Dale. 2002. The use of cottonseed meal with or without added soapstock in laying hen diets. J. Appl. Poult. Res. 11:127–133.

Dowd, M. K. 2003. Preparation of enantiomeric gossypol by crystallization. Chirality 15:486–493.

Eagle, E. 1949. Detoxification of cottonseed pigment glands with ferrous sulphate. Proc. Soc. Biol. Med. 72:444–446.

Gallup, W. D. 1928. The value of iron salts in counteracting the toxic effect of gossypol. J. Biol. Chem. 77:437–449.

Goodridge, A. G. 1968. Conversion of [U14-C] glucose into carbon dioxide, glycogen, cholesterol and fatty acids in liver slices from embryonic and growing chicks. Biochem. J. 108:655–661.

Goodridge, A. G., and E. G. Ball. 1967. Lipogenesis in the pigeon: In vivo studies. Am. J. Physiol. 213:245–249.

Heinstein, P., R. Widmaier, P. Wegner, and J. Howe. 1977. Biosynthesis of gossypol in cotton. Pages 313–337 in Recent Advances in Phytochemistry. F. A. Loewus and V. C. Runeckles. Plenum Press, New York, NY.

Heywang, B. W., H. R. Bird, and A. M. Altschul. 1950. The effect of pure gossypol on egg hatchability and weight. Poult. Sci. 29:916–920.

Heywang, B. W., H. R. Bird, and A. M. Altschul. 1954. Relationship between discolorations in eggs and dietary free gossypol supplied by different cottonseed products. Poult. Sci. 34:81–90.

Huang, L., D. K. Zheng, and Y. K. Si. 1987. Resolution of racemic gossypol. J. Ethnopharmacol. 20:13–20.

Kemmerer, A. R., B. W. Heywang, and M. G. Vavich. 1961. Effect of *Sterculia foetida* oil in gossypol discoloration in cold storage eggs and the mechanism of gossypol discoloration. Poult. Sci. 40:1045–1048.

Kemmerer, A. R., B. W. Heywang, M. G. Vavich, and E. T. Sheehan. 1966. Effect of iron sulphate on egg discoloration caused by gossypol. Poult. Sci. 45:1025–1028.

Leveille, G. A., E. K. O'Hea, and K. Chakrabarty. 1968. In vivo lipogenesis in the domestic chicken. Proc. Soc. Exp. Biol. Med. 128:398–401.

Lin, T. S., R. Schinazi, B. P. Griffith, E. M. August, B. F. Eriksson, D. K. Zheng, L. Huang, and W. H. Prusoff. 1989. Selective inhibition of human immunodeficiency virus type 1 replication by the (–) but not the (+) enantiomer of gossypol. Antimicrob. Agents Chemother. 33:2149–2151.

Liu, S. S., K. Kulp, Y. Sugimoto, J. Jiang, H. L. Chang, M. K. Dowd, P. Wan, and Y. C. Lin. 2002. The (–)-enantiomer of gossypol possesses higher anticancer potency than racemic gossypol in human breast cancer. Anticancer Res. 22:33–38.

Lordelo, M. M., A. J. Davis, M. C. Calhoun, M. K. Dowd, and N. M. Dale. 2005. Relative toxicity of gossypol enantiomers in broilers. Poult. Sci. 84:1376–1382.

Lordelo, M. M., A. J. Davis, J. L. Wilson, and N. M. Dale. 2004. Cottonseed meal diets improve body weight uniformity in broiler breeder pullets. J. Appl. Poult. Res. 13:191–199.

McMillan, M. 2000. Effects of processing cottonseed on plasma gossypol levels of cattle and sheep. MS Thesis. Angelo State Univ., San Angelo, TX.

Narain, R., C. M. Lyman, and J. R. Couch. 1957. High levels of free gossypol in hen diets: Effects on body weight, feed consumption, and egg production. Poult. Sci. 36:1651–1654.

Panigrahi, S. 1990. Ammonia and dietary cottonseed meal-associated brown yolk discolouration in hens' eggs. Trop. Sci. 30:325–342.

Panigrahi, S., and T. R. Morris. 1991. Effects of dietary cottonseed meal and iron-treated cottonseed meal in different laying hen genotypes. Br. Poult. Sci. 32:167–184.

Panigrahi, S., and V. E. Plumb. 1996. Effects on dietary phosphorus of treating cottonseed meal with crystalline ferrous sulphate for the prevention of brown yolk discolouration. Br. Poult. Sci. 37:403–411.

Panigrahi, S., V. E. Plumb, and D. H. Machin. 1989. Effects of dietary cottonseed meal, with and without iron treatment, on laying hens. Br. Poult. Sci. 30:641–651.

Phelps, R. A. 1966. Cottonseed meal for poultry: From research to practical application. World's Poult. Sci. J. 22:86–112.

Reid, B. L., S. Galaviz-Moreno, and P. M. Maiorino. 1984. A comparison of glandless and regular cottonseed meals for laying hens. Poult. Sci. 63:1803–1809.

Reid, B. L., S. Galaviz-Moreno, and P. M. Maiorino. 1987. Evaluation of isopropanol-extracted cottonseed meal for laying hens. Poult. Sci. 66:82–89.

Roberts, J. D., R. Stewart, and M. C. Caserio. 1971. Page 373 in Organic Chemistry: Methane to Macromolecules. W. A. Benjamin Inc., Menlo Park, CA.

SAS Institute. 2001. SAS User's Guide: Statistics. Version 8 ed. SAS Inst. Inc., Cary, NC.

Stipanovic, R. D., J. D. Lopez Jr., M. K. Dowd, L. S. Puckhaber, and S. E. Duke. 2006. Effect of racemic and (+)- and (–)-gossypol on the survival and development of *Helicoverpa zea* larvae. J. Chem. Ecol. 32:959–968.

Swensen, A. D., E. A. Fieger, and C. W. Upp. 1942. The nature of egg yolk discoloration produced by cottonseed meal. Poult. Sci. 21:374–378.

Walzem, R. L., R. J. Hansen, D. L. Williams, and R. L. Hamilton. 1999. Estrogen induction of VLDLy assembly in egg-laying hens. J. Nutr. 129:467S–472S.

Wang, N. G., L. F. Zhou, M. H. Guan, and H. P. Lei. 1987. Effects of (–) and (+) gossypol on fertility in male rats. J. Ethnopharmacol. 20:21–24.

Withers, W. A., and J. F. Brewster. 1917. Studies on cottonseed meal toxicity. II. Iron as an antidote. J. Biol. Chem. 15:161–166.